BURDEN OF VITAMIN B_{12} DEFICIENCY IN ECUADOR: ANALYSIS OF THE NATIONAL SURVEY OF HEALTH AND NUTRITION (ENSANUT-ECU)

A Thesis

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In Partial Fulfillment of the Requirements for the Degree of

Master of Science

by

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ABSTRACT

Background

Vitamin B_{12} deficiency (<148 pmol/L) and insufficiency (<221 pmol/L) constitute a global health problem, particularly in low-income settings and among children, women of reproductive age, and the elderly. There have been few Latin American national surveys to examine the burden of vitamin B_{12} deficiency and vitamin B_{12} insufficiency. The 2012 Ecuadorian National Survey of Health and Nutrition is a nationally-representative complex survey of the Ecuadorian population and provides the first national estimate of vitamin B_{12} status in Ecuador.

Methods

Vitamin B_{12} was measured in 16,152 people from 10 to 59 years of age. Association of vitamin B_{12} status and levels with different socio-demographic and anthropometric correlates (sex, age, area, region, economic quintile, education level, ethnicity, and body mass index) was explored with univariate and multivariate linear and logistic regression models using survey procedures in SAS 9.4.

Results

The prevalence of vitamin B_{12} deficiency and vitamin B_{12} insufficiency in the overall Ecuadorian population was 5.4% and 27.4%, respectively. The burden was highest among pregnant women (21% and 55%). In the multivariate analyses, sex, age, region, education level, economic quintile, and ethnicity were significantly associated with vitamin B_{12} status. Men had higher odds of vitamin B_{12} insufficiency when compared to women (OR 1.5, 95% CI 1.35-1.66). Individuals living in the coastal regions had higher natural logarithmically transformed vitamin B_{12} concentrations (β=0.21, $p<0.0001$) and lower odds of vitamin B_{12} deficiency (OR: 0.56, 95% CI: 0.44-0.72) and insufficiency (OR: 0.48, 95% CI 0.41-0.57) when compared to people living in the highlands.

Conclusions

Vitamin B_{12} insufficiency is a significant health problem in Ecuador. Information is lacking to estimate the burden of vitamin B_{12} deficiency and vitamin B_{12} insufficiency for significant risk groups such as infants, children <10y, pregnant and lactating women, and the elderly. Prospective studies are needed to determine the association of total vitamin B_{12} insufficiency with adverse health outcomes in this population.

BIOGRAPHICAL SKETCH

Cristina Güitrón was born in Guadalajara, México. She completed her bachelor's degree in human nutrition at Universidad Anáhuac in México City. After completing her studies, Cristina did a one-year internship in the nutrition department at the National Institute of Perinatology in México City and practiced as a nutritionist. Cristina obtained a Fulbright-García Robles scholarship in 2016 and enrolled in the Master of Science in nutrition program in Cornell University on the fall of 2017, with the mentorship of Dr. Julia Finkelstein. She is looking forward to starting the Ph.D. program in Cornell University on the fall of 2019 as part of the Finkelstein Research Group.

To my parents, whose loving support have led my way.

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LIST OF ABBREVIATIONS

BMI	Body Mass Index
CDC	Centers for Disease Control and Prevention
CI	95% Confidence Interval
ENSANUT-ECU	National Survey of Health and Nutrition of Ecuador
MMA	Methyl Malonic Acid
OR	Odds Ratio
SAM	S-adenosyl methionine
SAS	Statistical Analysis Software
WHO	World Health Organization
WRA	Women of Reproductive Age

INTRODUCTION

Biochemistry of vitamin B_{12}

Vitamin B_{12}, also known as cobalamin, is a water-soluble essential nutrient found exclusively in animal-source foods (1). In humans, vitamin B_{12} functions as a cofactor for two enzymes: methyl malonyl coenzyme A (CoA) mutase and methionine synthase (MS). Methyl malonyl CoA mutase is contained in the mitochondria and utilizes adenosylcobalamin to convert methyl malonyl CoA to succinyl CoA, a metabolite in the tricarboxylic acid cycle. The dysfunction or lack of methyl malonyl CoA mutase, or the absence of vitamin B_{12}, result in the accumulation of methyl malonic acid (2-4).

On the other hand, methionine synthase utilizes methylcobalamin to catalyze, in the cytoplasm, the remethylation of homocysteine to methionine. Methionine is then integrated in S-adenosyl methionine (SAM), a methyl donor required in more than a hundred reactions, some of which include DNA synthesis and repair and methylation.

In animal-source foods, vitamin B_{12} is bound to protein and must be released before absorption. This process is facilitated by the acid environment of the stomach. Intrinsic factor is produced by the parietal cells of the stomach and is essential for the absorption of vitamin B_{12} in the ileum. In circulation, vitamin B_{12} is bound to haptocorrin (70-90%) or transcobalamin (10-30%) (1, 4). Transcobalamin-bound vitamin B_{12} (also called holotranscobalamin) is the active form of vitamin B_{12} because it can enter the cells (5) via the transcobalamin receptor, CD320. (5, 6).

Deficiency or insufficiency of vitamin B_{12} lead to a deficit of one or both of its cofactor forms (i.e. adenosylcobalamin and methylcobalamin) intracellularly, which results in the impaired function of vitamin B_{12}-dependent enzymes. This disfunction, in turn, leads to impaired purine and thymidylate synthesis, as well as deficient methylation reactions due to insufficient SAM. These metabolic alterations result in a variety of signs and symptoms such as megaloblastic anemia and neurological, and cognitive abnormalities. Although the exact mechanisms that cause these manifestations have not been completely elucidated, faulty DNA synthesis and regulation(7, 8), and increased cellular stress and production of reactive oxygen species have been suggested as possible causes(9-11).

Vitamin B_{12} status is measured by circulating (total vitamin B_{12} and holotranscobalamin) and functional (methyl malonic acid and homocysteine) biomarkers (12, 13). Total vitamin B_{12} is the most commonly used biomarker for assessing vitamin B_{12} status (12). Vitamin B_{12} deficiency is and vitamin B_{12} insufficiency are commonly defined as total vitamin B_{12}< 148 pmol/L and <221 pmol/L, respectively (14).

Vitamin B_{12} deficiency

Vitamin B_{12} deficiency was first recognized in the context of pernicious anemia, an autoimmune disorder that impairs vitamin B_{12} absorption due to the destruction of the gastric cells that produce intrinsic factor (4). The classic presentation of vitamin B_{12} deficiency is megaloblastic anemia and neurologic alterations (10, 13) and this has been deemed relatively rare (10). On the other hand, vitamin B_{12} insufficiency (<221 pmol/L) in the absence of overt clinical symptoms (i.e. subclinical vitamin B_{12} deficiency) is more common (10) and has been identified as a potential health risk due to its increased susceptibility to vitamin B_{12} deficiency-associated adverse health outcomes (10, 12, 14-16).

There is evidence that a low vitamin B_{12} status during pregnancy is associated with adverse pregnancy outcomes and childhood development outcomes (12, 17), including intrauterine growth restriction, preeclampsia, and neural tube defects (18). Low vitamin B_{12} levels have also been associated with impaired cognitive function in children (17) and cognitive decline later in life (10, 19).

Risk factors of vitamin B_{12} deficiency

In high-income countries, vitamin B_{12} deficiency or insufficiency is most prevalent in the elderly (20) due to various causes such as decreased intake of animal products and malabsorption as a result of pernicious anemia, gastrointestinal surgery, chronic gastritis, and antacid drugs (10, 21). On the other hand, in low-income settings, the main cause of vitamin B_{12} deficiency is insufficient dietary intake resulting from a limited access to animal-source foods and exacerbated by gastrointestinal infections and parasitic infestations (4, 10, 20-22). In these settings, groups children, pregnant women, and women of reproductive age are particularly vulnerable (10, 20, 23).

Burden of vitamin B_{12} deficiency

The prevalence of vitamin B_{12} deficiency (<148 pmol/L) worldwide is widely variable according to age group and country setting. It ranges from as low as 1-4% in the general US (20, 24, 25) and Europe(10) to as high as 50% in Indian adults(10). Vitamin B_{12} insufficiency (<221 pmol/L) is more common, ranging from ~15% in the US(20) to 80% in India(10). In the US, the prevalence of vitamin B_{12} deficiency and vitamin B_{12} is higher in the elderly (≥70 y) compared to the general population (~6% and ~25%, respectively) (10, 20, 26)

Vitamin B_{12} deficiency or insufficiency is more common in Asian and African countries compared to European and North American settings(10, 20, 26). In Latin America several local and national studies have assessed the prevalence of vitamin B_{12} deficiency, ranging from 2.9% in Costa Rican Adult men to ~38% in Guatemalan women of reproductive age, and vitamin B_{12} insufficiency, ranging from 21% in Colombian children (<18y) to 68.2% in Guatemalan women of reproductive age.

In Ecuador, the DANS (27) and Endemain (28) National surveys aimed to assess maternal and child health from 1986 to 2004. Neither of these surveys measured serum vitamin B_{12}. More recently, the SABE(29) survey assessed the health of the elderly (≥60 y) Ecuadorian population and included an analysis of vitamin B_{12} levels. The prevalence of vitamin B_{12} deficiency (<148 pmol/L) was 13.5% in men and 6.6% in women, while, overall 31% of the elderly population was vitamin B_{12} insufficient (<221 pmol/L).

ENSANUT-ECU is the first Ecuadorian Nationally-representative survey to address the entire population (0-59y). There is currently no information on the national prevalence of vitamin B_{12} deficiency and insufficiency in Ecuador. Therefore, the objective of this analysis was to examine the burden of vitamin B_{12} deficiency and insufficiency in the Ecuadorian population.

METHODS

Study Population

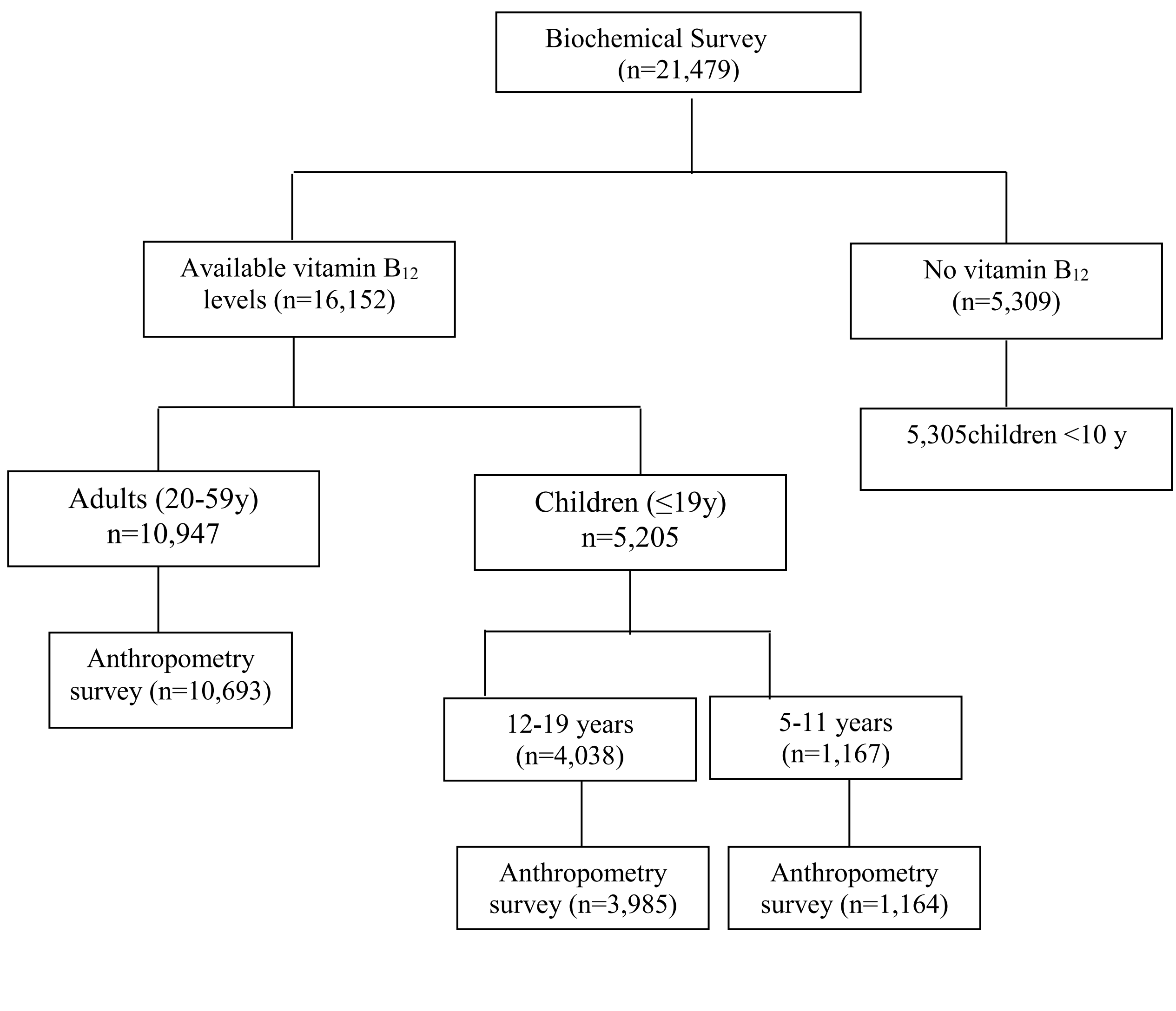
The National Health and Nutrition Survey from Ecuador (ENSANUT-ECU) is a nationally-representative complex survey of the Ecuadorian population that was conducted in 2012 to 92,502 individuals. The methods have been described in detail elsewhere (30-32). Briefly, the sample design was probabilistic, multistage, stratified, and clustered. The sample was representative at the national and regional levels.

Assessed variables included home and socio-demographic characteristics, sexual and reproductive behaviors and history, access to health care services, and health history. The nutrition component of the survey included biochemical analysis, food intake, anthropometric measurements, and breastfeeding and complimentary feeding practices. Informed consent was obtained from all participants.

A total of 19,949 homes were surveyed, which included 92,502 people. From each home, one participant was randomly selected from each group: one woman of reproductive age (12-49 years), one child younger than 5, one teenager (10-19 years), and one adult (20-59 years).

All included members in the household, except people older than 59 years of age and pregnant women, were eligible for anthropometric survey. A random subsample of 50% of the included homes was selected for the biochemistry survey (n=9,968 homes, 21,479 people). Vitamin B_{12} was analyzed on 16,152 samples from the target population of 10-59 years. The sample selection process is represented graphically in Figure 1. For this analysis, only the population with available vitamin B_{12} information was considered.

Figure 1 Sample Selection



Biochemical analyses

Total vitamin B_{12} was measured in the population 10 to 59 years of age by automated chemiluminescence (Immulite 2000).

Anthropometric measures

For weight measurement for both children and adults, an electronic Seca 874 scale with mother/child function (tare), 200 kg capacity, and 50g precision was used. Participants were asked to remove shoes and heavy clothing prior to the measurement. Children younger than 2 years were weighed in their mother's or primary caretaker's arms and with either no diaper or a clean and dry diaper.

Height was assessed with a Seca 217 stadiometer with a 20-205 cm range and 1 mm precision. Participants were asked to remove their shoes and hair accessories that could alter the measurement. Both parameters were measured in duplicate, and a third measurement was required when the difference between the first two was greater than 0.5 cm or 0.5 kg. The final value was the mean of the two closest measurements. Field anthropometry personnel were trained and standardized prior to data collection.

Vitamin B_{12} Status

Vitamin B_{12} deficiency was defined as vitamin B_{12} < 148 pmol/L and insufficiency was defined as vitamin B_{12} <221 pmol/L (14).

Definitions of variables

Age was categorized by two methods. Firstly, for comparison purposes, ENSANUT-ECU age categories are presented in table 1. These categories were defined for each decade from 20 to 60 years of age. Children were defined as ages 0-5 and 5-11, while adolescents were defined as 12-19 years of age. Age was also categorized in quintiles (Q1-Q5) for all analyses. Women of reproductive age were defined as non-pregnant females from 12 to 49 years of age.

Economic quintile was established from an economic score obtained by considering 42 variables pertaining to home characteristics and furnishings. Economic score has a normal distribution and quintiles were generated to categorize homes. The first economic quintile corresponds to the 20% homes with the lowest scores, while the fifth quintile includes the 20% of homes with the best economic conditions.

Area was defined as urban for populations of over 2000 people and rural otherwise. Region was defined by grouping of the subregions defined ENSANUT-ECU authors (Urban coast, rural coast, urban highlands, rural highlands, urban amazon, rural amazon, Galapagos, Quito, and Guayaquil). Urban and rural highlands, as well as Quito were classified as the highlands region. Urban coast, rural coast, and Guayaquil were categorized as coast. Urban and rural Amazon were defined as Amazon, while Galapagos was the only subregion that was not grouped with any others and remained as a region category.

Ethnicity was self-reported by survey participants or their caretakers in four categories: Indigenous, Montubio, Afro-Ecuadorian, and Mestizo/White/others.

Education level was self-reported into 10 categories: none, alphabetization center, preschool, elementary school, basic school, middle school, high-school, more than high school, college, graduate degree. For this analysis, these categories were grouped into four: none or incomplete elementary school, incomplete secondary, secondary, and more than secondary.

For children (≤19 years), weight adequacy was assessed using the World Health Organization (WHO) body mass index (BMI) (kg/m^2) for age z-scores (33, 34) with the 2007 (35) WHO downloadable SAS macro. Using the WHO-defined cut-offs (36), underweight was defined as a z-score < -2 standard deviations of the mean (SD). Normal weight was defined as a z-score ≥ -2 SD and ≤ +1 SD, overweight was defined as z-score > +1 SD and ≤ + 2 SD, and obesity was defined as z-score > + 2 SD.

For adults (> 19 years), BMI was categorized using WHO cut-offs. Underweight was defined as BMI <18.5 kg/m^2, normal weight was defined as BMI ≥18.5 and < 25 kg/m^2, overweight was defined as a BMI ≥25 and <30 kg/m^2, and obesity was defined as BMI ≥30 kg/m^2.

Statistical Analysis

Descriptive statistics were calculated for the outcomes of interest and exposure variables (sex, age, area, economic quintile, region, education level, ethnicity, height, weight, body mass index). Outliers were defined as those greater than quartile 3 + 3*IQR or lower than quartile 1- 3*IQR. Outliers were flagged but if deemed biologically plausible, were included in analyses. Descriptive statistics (mean, median, geometric mean) of vitamin B_{12} were calculated for each exposure variable of interest. A normal probability plot and Kolmogorov-Smirnov test showed that the distribution of vitamin B_{12} was not normal, hence vitamin B_{12} was natural logarithmically transformed for normality. Linear regression models were used to analyze the association between each exposure variable and the natural logarithmically transformed vitamin B_{12} concentrations. Logistic regression was conducted for categorical outcomes (i.e., vitamin B_{12} deficiency, vitamin B_{12} sufficiency/insufficiency) for each individual exposure.

In correlate analyses, variables were examined individually and then considered for inclusion in the complete model if their p-value was less than 0.20. Different versions of a single variable (for example, continuous and categorical age) were selected for inclusion in the final model according to their level of statistical significance. All variables with a p-value <0.20 where included in the model initially. A backward stepwise selection process was used to select the variables for the final model. The variable with the largest p-value was removed in a stepwise manner until all included variables included were significant at the 0.05 level. Final linear models were checked for normality using quantile-quantile plots and equal variance using predicted values and residual plots. Univariate and multivariate analyses were repeated for the children only (≤19y) and adult only (>19y) subgroups.

Survey procedures (i.e. Survey Means, Survey Frequency, Survey Logistic, and Survey Reg) from SAS 9.4 were used for statistical analyses. The survey-specific variables were defined as follows: cluster was the census section, strata were the area (urban or rural), and the sample weight was the individual weight assigned to each survey participants. All variables were obtained from the publicly available biochemistry data set of ENSANUT-ECU.

RESULTS

The characteristics of the whole ENSANUT-ECU sample (N=92,502), the biochemistry subsample (n=21,479), and the individuals with available vitamin B_{12} levels (n=16,152) are presented in Table 1. The main difference between these nested samples was the exclusion of people 60 years or older from the biochemical analysis and the fact that vitamin B_{12} was not analyzed in children younger than 10 years of age. Most of the Ecuadorian population (~80%) is of Mestizo, White, or other ethnicity and lives either in the coastal or highlands areas of the country (~94%). Women of reproductive age (n=8,996) represented 43.64% of the available vitamin B_{12} levels sample, while only 275 pregnant women were included in the biochemistry survey.

Descriptive statistics for vitamin B_{12} (arithmetic mean (SE), median (IQR) and geometric mean (SE)) for different socio-demographic and anthropometric characteristics are summarized in Table 2. Female participants had higher geometric mean vitamin B_{12} levels than male participants (286.02 vs 311.11 pmol/L, respectively). In terms of age, quintile 2 (16-24y) had the lowest mean vitamin B_{12} (geometric mean=281.17), while quintile 5 (>43y) had the highest mean vitamin B_{12} (geometric mean: 326.38).

No differences were found in vitamin B_{12} levels between urban and rural areas (geometric mean 287.06 and 286.33, respectively).

Table 1 Characteristics of the study population

Variable, Mean (SE) or population estimate, thousands (%)	Overall Sample			Biochemistry subsample			Vitamin B_{12} subsample		
	n missing (%)	n	Mean (SE) or Pop. Estimate in thousands (%)	n mising (%)	n	Mean (SE) or Pop. Estimate in thousands (%)	n missing (%)	n	Mean (SE) or Pop. Estimate in thousands (%)
Total		92502	15878		21479	13473		16152	10712
Female		47523	8139 (51.26)		12920	6791 (50.4)		10299	5647 (52.72)
Socio-demographic									
Age		92502	26.27 (0.14)		21479	24.49 (0.19)		16152	29.6 (0.17)
Age category	4888* (5)	87614							
0-5 years		11506	1787 (11.25)		2047	1375 (10.21)		0	-
5-11 years		15862	2528 (15.92)		4443	2005 (14.88)		1167$^{\Delta}$	623 (5.82)
12-19 years		16806	2791 (17.58)		4039	2478 (18.39)		4038	2477 (23.12)
20-29 years		15232	2615 (16.47)		3762	2654 (19.69)		3762	2654 (24.77)
30-39 years		12626	2216 (13.95)		3795	2178 (16.17)		3794	2178 (20.34)
40-59 years		9924	1834 (11.55)		2644	1710 (12.69)		2642	1706 (15.93)
50-59 years		5658	1127 (7.1)		749	1074 (7.97)		749	1074 (10.03)
*Age Quintiles***		92502			21479				
Q 1		20380	3201 (20.16)		5312	2751 (20.42)		3876	2262 (21.12)
Q 2		20102	3276 (20.63)		4831	2877 (21.35)		3144	2231 (20.83)
Q 3		18924	3244 (20.43)		3739	2604 (19.33)		3159	1963 (18.32)
Q 4		16932	2996 (18.87)		4546	2679 (19.88)		3768	2233 (20.84)
Q5		16164	3162 (19.91)		3051	2563 (19.02)		2205	2023 (18.89)
Life stage									
Pregnant women		782	115 (0.73)		275	144 (1.07)		275	144 (1.34)
WRA (12-49 y)		27902	4810 (30.3)		8998	4682 (34.75)		8996	4678 (43.64)

Table 1 (Continued)

Area[±]									
Urban		54092	58.48%		13067	60.84%		9959	61.66
Rural		38410	41.52%		8412	39.16%		6193	38.34
Region									
Highlands		41896	7409 (46.66)		9665	5942 (44.1)		7508	4859 (45.36)
Coast		24559	7647 (48.16)		6209	6779 (50.31)		4546	5296 (49.44)
Amazon		23346	800 (5.04)		4874	728 (5.41)		3588	539 (5.04)
Galápagos		2701	23 (0.14)		731	24 (0.18)		510	17 (0.16)
Economic status index	214 (0.2)			7 (0.1)			7		
Q1		24654	3218 (20.27)		5331	2935 (21.78)		3801	2217 (20.71)
Q2		21277	3274 (20.62)		4898	2761 (20.5)		3593	2090 (19.52)
Q3		18010	3095 (19.49)		4319	2618 (19.43)		3250	2055 (19.2)
Q4		15492	3160 (19.9)		3760	2539 (18.84)		2926	2102 (19.63)
Q5		12855	3100 (19.52)		3164	2615 (19.41)		2575	2242 (20.94)
Ethnicity	2412 (2.4)								
Indigenous		10949	968 (6.1)		2218	1156 (8.58)		1676	905 (8.45)
Afro-Ecuadorian		3662	671 (4.23)		783	955 (7.09)		577	758 (7.08)
Montubio		2889	860 (5.41)		776	1152 (8.55)		561	868 (8.1)
Mestizo/white, /others		72590	13002 (81.89)		17702	10210 (75.78)		13338	8181 (76.37)
Level of Education	13712 (13.5)			2047 (10.2)					
None/incomplete primary school		2705	495 (3.12)		333	269 (2)		289	243 (2.26)
Primary school/incomplete secondary		51817	8501 (53.54)		12696	7237 (53.71)		9463	5881 (54.9)
Secondary school		15772	2995 (18.86)		4189	2918 (21.66)		4188	2918 (27.24)
Greater than secondary school		8496	1749 (11.01)		2214	1674 (12.42)		2212	1670 (15.59)

Table 1 (Continued)

Anthropometric									
Adults (>19 y)		48328			10950			10947	
BMI (kg/m^2)	18622	29706	27.07 (0.06)	254 (1.9)	10696	27.01 (0.09)	254 (1.9)	10693	27.01 (0.09)
BMI category‡	18624 (40.4)	29704		256 (2)			256 (2)		
Underweight		313	63 (0.71)		131	98 (1.29)		131	98 (1.31)
Normal		10423	1805 (20.58)		3637	2589 (34)		3635	2588 (34.69)
Overweight		12404	2164 (24.66)		4462	3093 (40.62)		4462	3093 (41.46)
Obese		6564	1197 (13.65)		2464	1685 (22.12)		2463	1682 (22.54)
Overweight/obesity‡	18624 (40.4)			256 (2)			256 (2)		
Not overweight		10736	1868 (21.3)		3768	101 (35.28)		3766	2686 (36)
Overweight/obese		18968	3361 (38.31)		6926	160 (62.74)		6925	4775 (64)
Children (≤ 19 y)		44174			10529			5205	
BMI z-score Category$^{\Pi}$	19018 (42.9)	25156		1331 (15.7)			518 (10.2)		
Underweight		316	60 (0.85)		107	64 (1.09)		60	39 (1.26)
Normal		17855	2854 (40.17)		6654	3558 (60.74)		3437	2036 (65.67)
Overweight		4786	764 (10.75)		1664	868 (14.82)		852	501 (16.16)
Obese		2199	377 (5.31)		773	451 (7.7)		338	207 (6.68)
Overweight/obesity $^{\Pi}$	19018 (42.9)			1331 (15.7)			518 (10.2)		
Not overweight		18171	2915 (41.02)		6761	3622 (61.83)		3497	2075 (66.93)
Overweight/Obese		6985	1141 (16.06)		2437	1320 (22.53)		1190	708 (22.84)

*The 4888 individuals missing age category are older adults (>59y), that were included in demographic survey but excluded from all other ENSANUT analyses.
Δ18 children <10y with available vitamin B_{12} levels were excluded from this analysis.
**Cut-offs for age quantiles were defined for each subsample: Overall sample: 8y, 8-17y, 17-29y, 29-43y, >43y. Biochemistry subsample: <9y, 9-18y, 18-28y, 28-40y, >40y. Vitamin B_{12} subsample: <16y, 16-24y, 24-32y, 32-43y, >43y.
±Area (%) estimates are non-weighted.
‡Adult BMI (kg/m^2) was categorized by WHO cut-offs: underweight (<18.5), normal (18.5-24.9), overweight (25-29.9), and obese (≥30). Not overweight was BMI <25, overweight/obese was BMI ≥25.
Child BMI z-score was categorized by WHO cut-offs: underweight (<-2), normal (-2 - <1), overweight (1 - <2), and obese (>2). Not overweight was z-score <1, overweight/obese was z-score ≥1.

Table 2 Vitamin B_{12} concentrations by socio-demographic and anthropometric variables

Variable	n missing	n	Mean (SE)	Median (Q1, Q3)	Geometric Mean (SE)
Socio-demographic					
Sex		16152			
Male		5853	323.13 (5.67)	272.00 (204.24, 379.82)	286.02 (4.10)
Female		10299	351.25 (4.49)	301.32 (224.33,419.89)	311.11 (3.34)
Age Quintiles					
Q1 (<16y)		3876	326.08 (5.44)	282.22 (211.13,384.24)	292.58 (4.04)
Q2 (16-24y)		3144	317.54 (6.51)	268.78 (198.49,383.40)	281.17 (4.72)
Q3 (24-32y)		3159	324.46 (6.24)	274.51 (214.48,377.41)	290.14 (4.43)
Q4 (32-43y)		3768	349.60 (6.53)	295.40 (221.73,416.22)	308.18 (4.75)
Q5 (>43)		2205	373.97 (7.50)	318.28 (232.46,441.88)	326.38 (5.71)
Life stage					
Pregnant women		275	258.47 (14.71)	212.30 (153.39,303.67)	225.63 (10.49)
WRA (12-49 y)		8996	346.93 (4.76)	298.17 (224.17,411.90)	309.13 (3.52)
Area$^{\pm}$					
Urban		9959	323.28 (1.81)	275.22 (206.82,380.61)	287.06 (1.35)
Rural		6193	320.11 (2.19)	275.99 (208.86,378.63)	286.33 (1.66)
Region					
Highlands		7508	298.67 (3.64)	257.39 (198.72,345.47)	268.73 (2.73)
Coast		4546	376.61 (7.72)	319.42 (233.55,459.43)	332.25 (5.98)
Amazon		3588	309.91 (4.92)	264.01 (198.30,357.92)	275.87 (3.74)
Galápagos		510	408.72 (29.01)	355.76 (248.30,515.37)	356.58 (23.54)
Education					
None/less than primary		289	381.60 (19.10)	318.92 (239.49,446.61)	337.36 (13.00)
Primary/less than secondary		9463	337.48 (4.86)	286.68 (213.36,404.23)	299.51 (3.65)
Secondary		4188	343.43 (7.24)	286.96 (218.54,404.40)	301.94 (5.14)
Greater than secondary		2212	323.70 (6.56)	276.97 (207.94,381.58)	287.01 (4.89)
Economic quintile	7				
Q1		3801	340.98 (7.81)	289.95 (222.72,396.25)	304.08 (5.71)
Q2		3593	345.26 (8.20)	294.59 (216.66,414.04)	304.22 (6.07)
Q3		3250	345.74 (8.59)	283.37 (208.78,415.86)	301.93 (6.23)
Q4		2926	337.34 (7.59)	288.02 (212.02,403.27)	298.05 (5.80)
Q5		2575	321.11 (6.57)	276.64 (210.76,376.27)	287.25 (4.94)

Table 2 (Continued)

Ethnicity					
Indigenous		1676	307.32 (8.50)	266.32 (199.88,351.52)	276.58 (6.33)
Afro-Ecuadorian		577	404.39 (16.29)	351.01 (237.07,499.69)	352.97 (12.74)
Montubio		561	359.62 (13.67)	307.44 (231.15,439.50)	322.09 (10.28)
Mestizo, white, other		13338	332.89 (4.52)	281.64 (211.77,395.16)	294.64 (3.35)
Anthropometric					
Adults (>19 y)					
BMI Category	256				
Underweight (<18.5 kg/m^2)		131	378.38 (25.50)	303.58 (222.06,448.10)	329.04 (20.74)
Normal (18.5-24.9 kg/m^2)		3635	334.47 (5.54)	286.44 (218.50,392.17)	297.75 (4.11)
Overweight (25-29.9 kg/m^2)		4462	345.13 (5.96)	285.90 (214.87,412.15)	302.45 (4.36)
Obese (≥30 kg/m^2)		2463	363.90 (7.99)	307.63 (225.30,438.93)	320.97 (5.97)
Overweight (≥25 kg/m^2)		3766	336.07 (5.52)	286.92 (218.77,393.53)	298.84 (4.11)
Not overweight (<25 kg/m^2)		6925	351.74 (5.47)	293.35 (219.62,420.61)	308.85 (4.04)
Children (≤19 y)					
BMI z-score Category	518				
Underweight (z < -2)		60	311.69 (26.01)	266.98 (214.27,374.60)	274.46 (24.13)
Normal (z ≥ -2, <1)		3437	325.53 (5.81)	279.54 (210.50,387.67)	292.02 (4.35)
Overweight (z >1, <2		852	315.05 (11.54)	269.67 (206.94,367.06)	282.02 (8.54)
Obese (z ≥ 2)		338	327.68 (18.13)	281.71 (206.42,380.02)	295.17 (14.15)
Not overweight (z <1)		1190	318.74 (9.68)	273.31 (206.85,374.35)	285.80 (7.28)
Overweight/obese (z ≥ 1)		3497	325.27 (5.73)	279.33 (210.56,387.20)	291.68 (4.30)

±Area estimates are non-weighted.

Table 3 Prevalence of vitamin B_{12} deficiency and insufficiency

Variable	Vitamin B_{12} deficiency (<148 pmol/L)					Vitamin B_{12} insufficiency (<221 pmol/L)				
	n	N	N (thousands)	%	(95% CI)	n	N	N (thousands)	%	(95% CI)
National	970	16152	577	5.39	(4.85-5.97)	4881	16152	2932	27.38	(25.93-28.87)
Socio-demographic										
Sex										
Male	436	5853	299	5.89	(5.05-6.87)	2108	5853	1586	31.31	(29.24-33.47)
Female	534	10299	278	4.93	(4.28-5.67)	2773	10299	1346	23.84	(22.41-25.34)
Age Quintile										
Q1 (<16y)	211	3876	109	4.83	(3.91-5.94)	1265	3876	657	29.05	(26.73-31.48)
Q2 (16-24y)	246	3144	150	6.74	(5.57-8.14)	1158	3144	761	34.12	(31.5-36.84)
Q3 (24-32y)	176	3159	97	4.95	(4.01-6.09)	944	3159	540	27.51	(24.98-30.2)
Q4 (32-43y)	216	3768	114	5.09	(4.15-6.23)	1028	3768	547	24.49	(22.34-26.78)
Q5 (>43)	121	2205	106	5.26	(4.07-6.78)	486	2205	427	21.11	(18.7-23.75)
Life stage										
Pregnant women	64	275	31	21.26	(14.47-30.11)	166	275	80	55.41	(45.96-64.48)
WRA (12-49 y)	421	8996	191	4.09	(3.47-4.8)	2410	8996	1126	24.07	(22.52-25.68)
Area$^{\pm}$										
Urban	601	9959	--	6.03	(5.58-6.52)	3043	9959	--	30.56	(29.66-31.47)
Rural	369	6193	--	5.96	(5.4-6.58)	1838	6193	--	29.68	(28.55-30.83)
Region										
Highlands	530	7508	331	6.8	(6.01-7.69)	2528	7508	1666	34.28	(32.25-36.36)
Coast	171	4546	209	3.95	(3.22-4.83)	947	4546	1083	20.45	(18.52-22.51)
Amazon	251	3588	37	6.78	(5.71-8.02)	1311	3588	181	33.5	(30.9-36.2)
Galápagos	18	510	1	3.72	(2.03-6.74)	95	510	3	19.19	(13.2-27.04)

Table 3 (Continued)

Economic status index										
Q1	220	3801	93	4.21	(3.4-5.2)	1106	3801	539	24.31	(21.91-26.88)
Q2	211	3593	117	5.6	(4.43-7.07)	1088	3593	553	26.45	(23.64-29.47)
Q3	201	3250	111	5.39	(4.32-6.7)	1035	3250	587	28.56	(25.62-31.69)
Q4	185	2926	130	6.21	(5.07-7.58)	886	2926	609	29	(26.23-31.93)
Q5	153	2575	125	5.59	(4.44-7.03)	766	2575	644	28.74	(25.71-31.98)
Ethnicity										
Indigenous	130	1676	55	6.12	(4.6-8.1)	587	1676	288	31.78	(27.14-36.83)
Afro-Ecuadorian	26	577	30	3.96	(2.48-6.26)	115	577	137	18.07	(14.05-22.93)
Montubio	22	561	31	3.6	(2.18-5.9)	130	561	180	20.76	(16.66-25.57)
Mestizo, white, and others	792	13338	460	5.63	(5.02-6.3)	4049	13338	2328	28.45	(26.86-30.1)
Level of Education										
None/incomplete primary school	11	289	4	1.54	(0.75-3.12)	61	289	45	18.43	(13.35-24.88)
Primary school/incomplete secondary	548	9463	305	5.18	(4.51-5.94)	2915	9463	1637	27.84	(26.12-29.63)
Secondary school	265	4188	162	5.54	(4.63-6.61)	1232	4188	756	25.9	(23.7-28.24)
Greater than secondary school	146	2212	107	6.41	(4.94-8.29)	673	2212	495	29.63	(26.44-33.03)
Anthropometric										
Adults (>19 y)	603	10691				2940	10691			
BMI Category										
Underweight (<18.5 kg/m^2)	10	131	7	6.65	(2.58-16.11)	30	131	19	19.69	(11.33-31.99)
Normal (18.5 - <25.0 kg/m^2)	204	3635	137	5.29	(4.24-6.59)	1008	3635	669	25.84	(23.56-28.25)
Overweight (25.0 - <30.0 kg/m^2)	271	4462	173	5.6	(4.66-6.71)	1277	4462	837	27.06	(24.84-29.4)
Obese (≥30.0 kg/m^2 kg/m^2)	118	2463	72	4.31	(3.22-5.74)	625	2463	372	22.12	(19.7-24.74)
Not overweight (<25 kg/m^2)	214	3766	143	5.34	(4.31-6.6)	1038	3766	688	25.61	(23.36-28)
Overweight/obese (≥25 kg/m^2)	389	6925	246	5.14	(4.42-5.98)	1902	6925	1209	25.32	(23.56-27.16)

Table 3 (Continued)

Children (≤19 years	265	4687				1570	4687			
Underweight (z < -2)	4	60	4	11.07	(3.2-31.96)	18	60	15	37.54	(21.64-56.68)
Normal (z ≥ -2, <1)	191	3437	93	4.57	(3.64-5.71)	1124	3437	593	29.12	(26.71-31.66)
Overweight (z >1, <2	51	852	31	6.24	(4.12-9.33)	315	852	167	33.23	(28.31-38.54)
Obese (z ≥ 2)	19	338	7	3.48	(1.98-6.06)	113	338	60	29.03	(21.96-37.3)
Not overweight (z<1)	195	3497	97	4.69	(3.75-5.85)	1142	3497	608	29.28	(26.89-31.8)
Overweight /obese (z ≥ 1)	70	1190	38	5.43	(3.79-7.72)	428	1190	227	32	(27.92-36.38)

±Area estimates are unweighted.

The prevalence of vitamin B_{12} deficiency (<148 pmol/L) and vitamin B_{12} insufficiency (<221 pmol/L) by different socio-demographic and anthropometric variables is presented in Table 3. The overall prevalence of vitamin B_{12} deficiency was 5.4%. (95% CI: 4.9%-6%), while vitamin B_{12} insufficiency was 27.4%. (95% CI: 25.9%-29%). The highest prevalence of vitamin B_{12} insufficiency was observed among pregnant women (55.4%. CI: 46-64.5), compared to non-pregnant women of reproductive age (12-49y) (24.1%. CI: 22.5-25.7).

The correlates of natural logarithmically transformed vitamin B_{12} levels are presented in Table 4a. Men had lower vitamin B_{12} levels compared to women (β= -0.084, $p<0.0001$). Similarly, women of reproductive age (12-49 years) and pregnant women had lower vitamin B_{12} levels compared to women who were younger than 12 or older than 49 years (β=0.099, p=0.0005) and non-pregnant, respectively (β= -0.312, $p<0.0001$).

Both region and ethnicity were significantly associated with vitamin B_{12} levels, while no significant association was observed for vitamin B_{12} levels and area (i.e. rural or urban). Univariate associations were also measured for children (10-19 y, n=5,205) and adults (>19y, n=10,947) separately (appendix tables 2 and 3).

The associations of sociodemographic and anthropometric factors with the odds of vitamin B_{12} deficiency or insufficiency are summarized in Table 4b. Individuals in the coast has lower odds of vitamin B_{12} deficiency 0.56 (95% CI: 0.44 , 0.72) and insufficiency (0.49, 95%CI 0.42-0.57) when compared to individuals in the highland regions.

The tables of the subgroup analyses for children (10-19 y) and adults (>19 y) are included in appendix tables 4 and 5.

Table 4a Correlates of natural logarithmically transformed vitamin B_{12} concentrations

Variables	Beta	SE	P value
Socio-demographic			
Sex			<0.0001
Male	-0.08	0.01	<0.0001
Female	Reference		
Age, years	0.003	0.001	<0.0001
Age Quintiles			<0.0001
Q1 (<16y)	0.01	0.02	0.58
Q2 (16-24y)	-0.03	0.02	0.07
Q3 (24-32y)	Reference		
Q4 (32-43y)	0.06	0.02	0.0003
Q5 (>43)	0.12	0.02	<0.0001
Life stage			
Pregnant women	-0.31	0.05	<0.0001
WRA (12-49y) vs. Women non WRA	-0.1	0.03	0.0005
Area$^{\pm}$			0.73
Urban	Reference		
Rural	-0.01	0.02	0.73
Region			<0.0001
Highlands	Reference		
Coast	0.21	0.02	<0.0001
Amazon	0.03	0.02	0.12
Galápagos	0.28	0.07	<0.0001
Economic status index			0.15
Q1	Reference		
Q2	0.001	0.02	0.98
Q3	-0.01	0.03	0.79
Q4	-0.02	0.03	0.45
Q5	-0.057	0.03	0.02

Table 4 (Continued)

Ethnicity			
			<0.0001
Indigenous	-0.06	0.02	0.01
Afro-Ecuadorian	0.18	0.04	<0.0001
Montubio	0.09	0.03	0.01
Mestizo, white, and others	Reference		
Level of Education			0.001
None/incomplete primary school	Reference		
Primary school/incomplete secondary	-0.119	0.04	0.002
Secondary school	-0.111	0.04	0.01
Greater than secondary school	-0.162	0.04	0.0001
Anthropometric			
Adults (>19 years)			
BMI (kg/cm^2)	0.003	0.001	0.01
BMI category			0.0003
Underweight (<18.5 kg/m^2)	0.100	0.06	0.11
Normal (18.5 - <25.0 kg/m^2)	Reference		
Overweight (25.0 - <30.0 kg/m^2)	0.016	0.01	0.29
Obese (≥30.0 kg/m^2)	0.075	0.02	<0.0001
Overweight (≥25.0 vs not)	0.033	0.01	0.01
Children (≤19y)			
BMI z-score Category			0.62
Underweight (z < -2)	-0.062	0.09	0.49
Normal (z ≥ -2, <1)	Reference		
Overweight (z >1, <2	-0.035	0.03	0.27
Obese (z ≥ 2)	0.011	0.05	0.83
Overweight or obese (z ≥ 1 vs not)	-0.020	0.03	0.47

±Area estimates are unweighted.

Table 4b Correlates of vitamin B_{12} deficiency and insufficiency

Variable	Vitamin B_{12} deficiency (<148 pmol/L)				Vitamin B_{12} insufficiency (<221 pmol/L)			
	n/N	OR	95% CI	P	n/N	OR	95% CI	P
Socio-demographic								
Sex	970/16152			0.1	4881/16152			<0.0001
Male	436/5853	1.21	(0.97, 1.51)	0.1	2108/5853	1.46	(1.32, 1.61)	<0.0001
Female		Reference						
Age, years		0.1	(0.99, 1.01)	0.1		0.99	(0.98, 0.99)	<0.0001
Age quintiles				0.09				<0.0001
Q1 (<16y)	211/3876	0.98	(0.73, 1.31)	0.87	1265/3876	1.08	(0.92, 1.26)	0.34
Q2 (16-24y)	246/3144	1.39	(1.03, 1.87)	0.03	1158/3144	1.37	(1.17, 1.6)	<0.0001
Q3 (24-32y)	176/3159	Reference			944/3159	Reference		
Q4 (32-43y)	216/3768	1.03	(0.77, 1.38)	0.84	1028/3768	0.86	(0.73, 1)	0.05
Q5 (>43)	121/2205	1.07	(0.75, 1.52)	0.72	486/2205	0.71	(0.58, 0.86)	0.0004
Life stage								
Pregnant women	64/275	6.03	(3.63, 10.01)	<0.0001	166/275	3.94	(2.67, 5.82)	<0.0001
WRA (12-49y) not pregnant	421/8996	0.58	(0.37, 0.9)	0.02	2410/8996	1.53	(1.19, 1.97)	0.001
Area‡				0.84				0.24
Urban	601/9959	Reference			3043/9959	0.96	(0.9, 1.03)	0.24
Rural	369/6193	0.99	(0.86, 1.13)	0.84	1838/6193	Reference		
Region	970/16152			<0.0001				<0.0001
Highlands	530/7508	Reference			2528/7508	Reference		
Coast	171/4546	0.56	(0.44, 0.72)	<0.0001	947/4546	0.49	(0.42, 0.57)	<0.0001
Amazon	251/3588	1	(0.8, 1.25)	0.97	1311/3588	0.97	(0.83, 1.12)	0.65
Galápagos	18/510	0.53	(0.28, 1)	0.05	95/510	0.46	(0.29, 0.72)	0.01

Table 4b (Continued)

Economic status index	970/16145			0.11	4881/16145			0.09
Q1	220/3801	Reference			1106/3801	Reference		
Q2	211/3593	1.35	(0.98, 1.86)	0.07	1088/3593	1.12	(0.92, 1.36)	0.25
Q3	201/3250	1.3	(0.95, 1.77)	0.10	1035/3250	1.25	(1.02, 1.52)	0.03
Q4	185/2926	1.51	(1.11, 2.05)	0.01	886/2926	1.27	(1.05, 1.54)	0.01
Q5	153/2575	1.35	(0.97, 1.87)	0.08	766/2575	1.26	(1.03, 1.54)	0.03
Ethnicity	970/16152			0.14				<0.0001
Indigenous	130/1676	1.09	(0.8, 1.5)	0.58	587/1676	1.17	(0.93, 1.48)	0.19
Afro-Ecuadorian	26/577	0.69	(0.42, 1.13)	0.14	115/577	0.55	(0.41, 0.75)	0.0001
Montubio	22/561	0.63	(0.37, 1.07)	0.09	130/561	0.66	(0.5, 0.87)	0.004
Mestizo, white, and others	792/13338	Reference			4049/13338	Reference		
Level of Education	970/16152			0.003				0.01
None/incomplete primary school	11/289	Reference			61/289	Reference		
Primary school/incomplete secondary	548/9463	3.5	(1.68, 7.27)	0.001	2915/9463	1.71	(1.17, 2.5)	0.01
Secondary school	265/4188	3.75	(1.77, 7.93)	0.001	1232/4188	1.55	(1.04, 2.31)	0.03
Greater than secondary school	146/2212	4.39	(2.02, 9.5)	0.0002	673/2212	1.86	(1.24, 2.81)	0.003
Anthropometric								
Adults (>19 y)	603/10691				2940/10691			
BMI		0.98	(0.96, 1.01)	0.20		0.96	(0.98, 1)	0.06
BMI Category				0.48				0.02
Underweight (<18.5 kg/m^2)	10/131	1.28	(0.458, 3.55)	0.64	30/131	0.70	(0.37, 1.36)	0.29
Normal (18.5 - <25.0 kg/m^2)	204/3635	Reference			1008/3635	Reference		
Overweight (25.0 - <30 kg/m^2)	271/4462	1.06	(0.79, 1.44)	0.70	1277/4462	1.07	(0.92, 1.24)	0.41
Obese (≥30 kg/m^2)	118/2463	0.81	(0.55, 1.19)	0.27	625/2463	0.82	(0.68, 0.97)	0.02
Overweight or obese (≥25 kg/m^2 vs not)	389/6925	0.96	(0.73, 1.27)	0.78	1902/6925	0.99	(0.86, 1.13)	0.82

Table 4b (Continued)

Children (≤19 years)	265/4687				1570/4687			
BMI z-score Category				0.18				0.37
Underweight (z < -2)	4/60	2.6	(0.68, 9.95)	0.16	18/60	1.463	(0.67, 3.22)	0.34
Normal (z ≥ -2, <1)	191/3437	Reference			1124/3437	Reference		
Overweight (z >1, <2	51/852	1.39	(0.85, 2.28)	0.19	315/852	1.211	(0.95, 1.55)	0.12
Obese (z ≥ 2)	19/338	0.75	(0.4, 1.41)	0.38	113/338	0.996	(0.67, 1.49)	0.98
Overweight or obese (z ≥1) vs not	70/1190	1.17	(0.75, 1.82)	0.49	428/1190	1.137	(0.92, 1.41)	0.25

‡Area estimates are unweighted

Findings of multivariate analyses are presented in Tables 5 a-c. When compared to people in the highlands, individuals living in coastal regions had higher vitamin B_{12} concentrations (β=0.21, p <0.0001) and lower odds of vitamin B_{12} deficiency (OR: 0.56, 95% CI: 0.44-0.72) and vitamin B_{12} insufficiency (OR: 0.48, 95% CI: 0.41-0.57). Compared to women, men had lower levels of vitamin B_{12} (β= -0.09, p <0.0001) and higher odds of vitamin B_{12} insufficiency (OR: 1.5, 95% CI: 1.35-1.66).

In terms of age, age quintiles 4 (32-43 y) and 5 ($\geq$ 43 y) had statistically significant higher vitamin B_{12} concentrations (β=0.06, p=0.0002, and β=0.11, p <0.0001) when compared to age quintile 3 (24-32 y). Similarly, compared to quintile 3, individuals in age quintiles 4 and 5 had lower odds of vitamin B_{12} insufficiency (OR: 0.84, 95% CI: 0.72-0.98 and OR: 0.70, 95% CI: 0.58-0.85, respectively), while individuals in age quintile 2 (16-24 y) had higher odds of vitamin B_{12} insufficiency (OR: 1.33, 95% 1.14-1.56).

Regarding ethnicity, Afro-Ecuadorian ethnicity was associated with higher vitamin B_{12} levels (β=0.13, p <0.0001) and lower odds of vitamin B_{12} insufficiency (OR: 0.65, 95% CI: 0.49-0.88).

Analyses within adult (>19y) and children ($\leq$19y) subgroups and children subgroups are included in appendix tables 6a-c and 7a-c, respectively. The main differences between these subgroup models and the overall models were the inclusion of the area (i.e. urban, rural) correlate in the adult insufficiency model and that neither ethnicity nor education level were kept for any of the children-only models.

Table 5a Multivariate model for natural logarithmically transformed vitamin B_{12}

Variable	F value	p-value	Beta	SE	p-value
Sex	62.600	<0.0001			
Male			-0.09	0.01	<0.0001
Female			Reference		
Age quintile	14.250	<0.0001			
Q1 (<16y)			0.01	0.02	0.76
Q2 (16-24y)			-0.03	0.02	0.13
Q3 (24-32y)			Reference		
Q4 (32-43y)			0.06	0.02	0.0002
Q5 (>43)			0.11	0.02	<0.0001
Ethnicity	5.48	0.001			
Indigenous			0.01	0.02	0.76
Afro-Ecuadorian			0.13	0.03	<0.0001
Montubio			-0.01	0.03	0.86
Mestizo/white/others			Reference		
Region	37.27	<0.0001			
Highlands			Reference		
Coast			0.21	0.02	<0.0001
Amazon			0.03	0.02	0.19
Galápagos			0.28	0.07	<0.0001
Level of Education	2.66	0.05			
None/incomplete primary school			Reference		
Primary school/incomplete secondary			-0.04	0.04	0.27
Secondary school			-0.06	0.04	0.15
Greater than secondary school			-0.09	0.04	0.02

Table 5b Multivariate model for vitamin B_{12} deficiency

Variable	OR	95% CI		p-value
Region				<0.0001
Coast	0.56	0.44	0.72	<0.0001
Amazon	1.01	0.82	1.27	0.92
Galápagos	0.53	0.28	0.99	0.05
Highlands	Reference			
Level of Education				0.002
None/incomplete primary school	Reference			
Primary school/incomplete secondary	3.58	1.72	7.46	0.0007
Secondary school	3.92	1.85	8.27	0.0004
Greater than secondary school	4.47	2.06	9.69	0.0002

Table 5c Multivariate model for vitamin B_{12} insufficiency

Variable	OR	95% CI		p-value
Sex				<0.0001
Male	1.5	1.35	1.66	<0.0001
Female	Reference			
Age quintile				<0.0001
Q1 (<16y)	1.01	0.87	1.19	0.86
Q2 (16-24y)	1.33	1.14	1.56	0.0004
Q3 (24-32y)	Reference			
Q4 (32-43y)	0.84	0.72	0.98	0.03
Q5 (>43)	0.70	0.58	0.85	0.0003
Region				<0.0001
Coast	0.48	0.41	0.57	<0.0001
Amazon	0.99	0.84	1.16	0.88
Galápagos	0.46	0.28	0.73	0.001
Highlands	Reference			
Economic quintile				0.043
Q1	Reference			
Q2	1.18	0.96	1.43	0.11
Q3	1.33	1.07	1.65	0.004
Q4	1.31	1.07	1.59	0.01
Q5	1.11	0.89	1.38	0.26

Table 5c (Continued)

Ethnicity				0.05
Indigenous	0.97	0.76	1.24	0.82
Afro-Ecuadorian	0.65	0.5	0.88	0.01
Montubio	0.95	0.70	1.28	0.73
Mestizo/white/others	Reference			

DISCUSSION

Burden of vitamin B_{12} deficiency and vitamin B_{12} insufficiency

In Ecuador, the prevalence of vitamin B_{12} deficiency is 5.4% (<148 pmol/L), while 27.4% of the population has vitamin B_{12} insufficiency (<221 pmol/L). The prevalence of vitamin B_{12} deficiency and vitamin B_{12} insufficiency was highest among pregnant women (21.3% and 55.4%, respectively), individuals in the highands regions (6.8% and 34.3%, respectively), and individuals between the ages of 16 and 24 years (6.7% and 34.1%, respectively), compared to the general population.

The prevalence of vitamin B_{12} deficiency and insufficiency in Ecuador is similar to the reported estimates for other Latin American countries(37). For Ecuadorian women of reproductive age (12-49 y) the prevalence of vitamin B_{12} deficiency (4%) is similar to the estimate for Costa Rican (5%) and Argentinian (3.4%) women of similar age, but lower than the comparable estimate for Mexican women ages 20-49y (8.5%)(38) Pregnant women have the highest prevalence of vitamin B_{12} deficiency in Ecuador (21%). This estimate is comparable with the 18.9% prevalence of vitamin B_{12} deficiency in a sample of pregnant women (n=1,781) from a nationally-representative survey from Colombia, but lower than the 30.9% estimate of vitamin B_{12} deficiency in Argentinian women (n=1,612) (Appendix table 1).

The 27% overall prevalence of vitamin B_{12} insufficiency in Ecuador represents an important public health problem. It has been suggested that low vitamin B_{12} levels, even in the absence of the classical clinical presentation of hematological or neurological symptoms, is associated with adverse health outcomes such as cognitive impairment in children(17) and older adults (19)and depression(10). This is especially a problem for developing infants(39), whose vitamin B_{12} status is largely determined by maternal vitamin B_{12} status(40, 41). The high burden of vitamin B_{12} deficiency during pregnancy in Ecuador and other Latin American countries also represents a public health problem because of the association of low vitamin B_{12} levels and adverse pregnancy outcomes (12), such as neural tube defects (18).

Socio-demographic correlates of vitamin B_{12} status

In Ecuador, men had significantly lower vitamin B_{12} concentrations (β= -0.09, p<0.0001) and higher odds for vitamin B_{12} insufficiency (OR: 1.5, 95% CI: 1.35-1.66) compared to women. This finding is inconsistent with the literature, that suggests that women have a higher risk of vitamin B_{12} deficiency(10, 20, 26, 42). It is unlikely that this observation can be explained by diet alone, since the ENSANUT-ECU report (32) described vitamin B_{12} intake was stlightly lower in women than men.

Likewise, vitamin B_{12} concentrations were higher among individuals in age quintiles 4 (32-43y) (β=0.06, p=0.0002) and 5 (≥43y) (β=0.11, p <0.0001), compared to age quintile 3 (24-32y), while odds of vitamin B_{12} insufficiency were higher for individuals in age quintile 2 (16-24y) (OR: 1.33, 95% CI: 1.14-1.56) and lower for indivuals in age quintiles 4 (OR:0.84, 95% CI: 0.72-0.98) and 5 (OR: 0.7, 95% CI: 0.58-0.85), compared to age quintile 3. This is also inconsistent with current literature that suggests that older adults have an increased risk of vitamin B_{12} deficiency and vitamin B_{12} insufficiency due to inadequate intake and impaired absorption(10, 20). However, the age range is not entirely comparable since older adults are generally described as older than 60-65 y and people older than 59 years were not included in the biochemical survey of ENSANUT-ECU.

Furthermore, the observation in ENSANUT-ECU that a lower economic quintile and/or education level were associated with higher vitamin B_{12} levels and improved vitamin B_{12} status is also inconstintent with the literature(10, 20, 21). A national survey from Argentina(43), found that higher economic status was associated with improved vitamin B_{12} status in pregnant women, while no association was observed between economic status and vitamin B_{12} levels or status in non-pregnant women ages 10-49. In Costa Rica (44), higher education level was associated with a better vitamin B_{12} status in women of reproductive age (15-44 y) but no association was observed in men, women ages 45-64, or the elderly (≥65 y). It has been suggested that limited access to animal-source foods in low-resource settings is associated with decreased vitamin B_{12} intake and, consequently, poor vitamin B_{12} status(10, 21).

In addition, compared to individuals in the highlands region, individuals in the coast and Galápagos regions had higher vitamin B_{12} concentrations (β=0.21, p<0.0001 and β=0.28, p <0.0001) and lower odds of vitamin B_{12} deficiency (coast OR: 0.56, 95% CI: 0.44-0.72, Galápagos OR: 0.52, 95% CI: 0.28-0.99) and insufficiency (coast OR: 0.48, 95% CI: 0.41-0.57, Galápagos OR: 0.46, 95% CI: 0.28-0.74). Higher fish and shellfish intake in the coastal regions(32) may be associated with this finding, but this possibility has not been explored.

On the other hand, there was no difference in vitamin B_{12} levels or status between urban and rural areas in Ecuador. This was consistent with findings from a national survey from Costa Rica (44), while a rural setting was associated with lower vitamin B_{12} levels in a nationally representative sample of Colombian pregnant women (45).

Although, in the univariate analyses, the Indigenous population in Ecuador had lower vitamin B_{12} levels and people of Montubio and Afro-Ecuadorian ethnicity had higher vitamin B_{12} levels when compared to Mestizo or white individuals, this association was only significant for the Afro-Ecuadorian population in the multivariate analyses. Ethnicity, specifically indigenous, was a significant correlate of vitamin B_{12} deficiciency in pregnant women in Colombia(45) and Mexico(38).

Body Mass Index (BMI) was not associated with vitamin B_{12} status in the multivariate analyses. Although there were some associations between BMI, BMI WHO categories, and BMI z-score categories and vitamin B_{12} status and levels in the univariate analyses, none of the associations remained significant in any of the multivariate models.

Strengths

ENSANUT-ECU is the first nationally-representative survey in Ecuador to assess vitamin B_{12} status in the overall population. The representative sampling allows for the estimation of the first national-level prevalence estimate of vitamin B_{12} deficiency and vitamin B_{12} insufficiency, as well as the prevalence of vitamin B_{12} deficiency and vitamin B_{12} insufficiency various key risk groups, such as pregnant women and women of reproductive age.

Limitations

There are several limitations that constrain the interpretation of the findings of this analysis. Firstly, given that this is a cross-sectional study, we cannot infer temporality or causality of the observed associations. Furthermore, data was not available for children younger than 10 years, lactating women, or adults older than 60 years, all of which are groups at risks of vitamin B_{12} deficiency. Similarly, as the sample of pregnant women was small, the estimates of vitamin B_{12} deficiency and vitamin B_{12} insufficiency in this subgroup could be inaccurate.

Moreover, vitamin B_{12} measurement was limited to one time point and no functional vitamin B_{12} biomarkers were considered. Another limitation is the lack of consideration folate status and functional outcomes (ie. anemia, cognitive abnormalities, growth) in this analysis, as well as dietary intake of animal-source foods and vitamin B_{12} and prescence of infections that may affect vitamin B_{12} absorption. Finally, the pregnancy variable was self-reported and women did not know if they were or not pregnant were classified as not pregnant, which could have altered the estimates of vitamin B_{12} deficiency and vitamin B_{12} insufficiency in these subgroups.

Future research recommendations

ENSANUT-ECU is the first survey to estimate vitamin B_{12} status at the National level in Ecuador. More information is needed to estimate the burden of vitamin B_{12} deficiency and vitamin B_{12} insufficiency for key risk groups in Ecuador; particularly infants, children, pregnant women, lactating women, and the elderly. Further national surveys could oversample pregnant and lactating women to have more accurate estimates for these subgroups. Additionally, the use of a functional biomarker for vitamin B_{12} status (ie. MMA) in a future Ecuadorian National survey would be valuable to better estimate subclinical and functional insufficiencies of vitamin B_{12} in this population (14, 46, 47). The consideration of the folate status and functional outcomes of vitamin B_{12} deficiency and vitamin B_{12} insufficiency (eg. anemia, neurological and cognitive abnormalities) is also of interest for future analyses.

Finally, although overall the prevalence of vitamin B_{12} deficiency was low, the high prevalence of vitamin B_{12} insufficiency in Ecuador warrants further research in this population. Prospective studies with functional biomarkers are needed to understand the role of vitamin B12 in the development of functional outcomes to inform the development of interventions.

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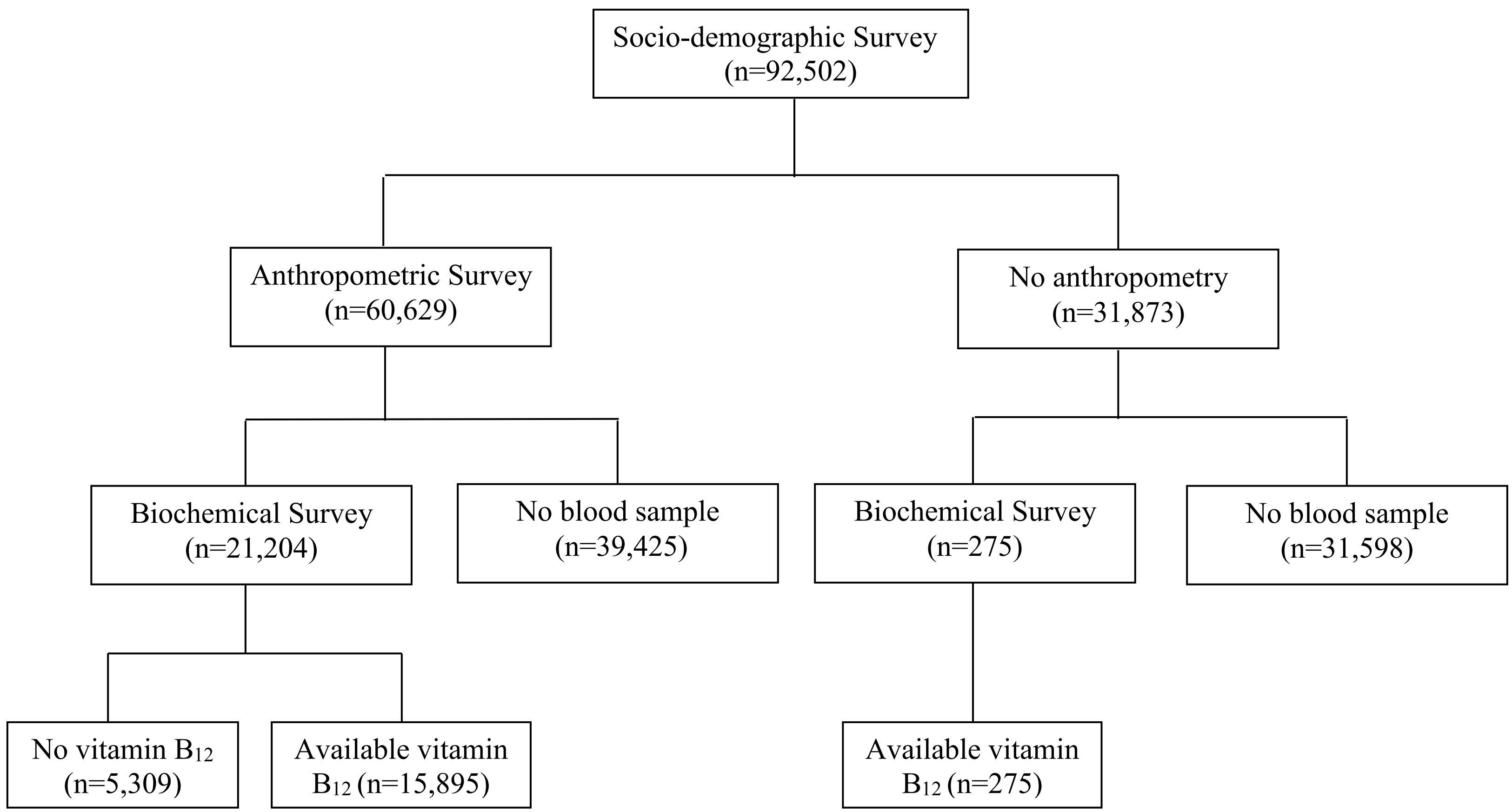
APPENDIX

Appendix Figure 1 ENSANUT-ECU sample selection



Appendix Table 1 Prevalence of vitamin B_{12} deficiency and insufficiency in national surveys from Latin American Countries

Country (Year)	Survey	Definition of vitamin B_{12} status	Population	Prevalence of deficiency	Prevalence of insufficiency	Other findings
Argentina (2007)	ENNyS(43)	Deficiency: <150 pg/mL (110.7 pmol/L) Insufficiency: <200 pg/mL (147.6 pmol/L)	N=6,605 women 10-49y	3.4%	11.9%	No statistically significant differences of median vitamin B_{12} between different levels of economic status.
			N= 1,612 pregnant women	18.2%	30.9%	25.8% of women ages 10-29 had inadequate vitamin B_{12} intake (<2.4 µg/d). Inadequate intake of vitamin B_{12} was significantly higher in indidivuals in households with unsatisfied basic needs compared to without unsatisfied basic needs (7.7%. 95%CI: 6-9.8%) ,(3.1%. 95% CI: 2.5%-3.9%), respectively.
Chile (2009)	ENS(48)	Deficiency: ≤200 pg/mL (147.6 pmol/L) Insufficiency: <299.5 pg/mL (221 pmol/L)	N=817 adults ≥65 y	8.1%	33.6%	For the ≥75y age group, women had a significantly lower prevalence of insufficiency than men (26.2% vs 45.3%, p=0.037). No other age sex differences were observed.

Appendix table 1 (Continued)

Colombia (2010)	ENSIN(45, 49, 50)	Deficiency: <148 pmol/L Insufficiency: <221 pmol/L	N=7243 children <18y	2.8%	18.2%	Overall :6.6% deficiency, 22.5% insufficiency. Serum vitamin B_{12} was 12 pmol/L higher in girls than boys (p=0.004) and 21 pmol/L lower in children from households with severe food insecurity (p=0.003) compared to children from households without food insecurity.
			N=1,781 pregnant women	18.9%	41.3%	In pregnant women, university education of the household head was associated with 24 pmol/L higher vitamin B_{12} compared to houshold head having less than primary education. Living in the National territories, the Eastern or the Pacific regions was associated with significantly lower serum vitamin B_{12} levels than living in other regions.

Appendix table 1 (Continued)

Costa Rica (2008)	ENN(44)	Deficiency: <193 pg/mL (142.4 pmol/L)	n=600 Women 15-44y	4.8%	N/A	Overall prevalence of deficiency was 4.6%
			n=750 women 45-64y	6.4%		
			n=600 men 20-64y	2.9%		
			n=500 ≥65y	5.3%		
Ecuador (2011)	SABE II(29)	Deficiency: <200 pg/mL (148 pmol/L)	n=1,065 men ≥60y	13.5% men		Adults represented the coastal and Andes (highlands) regions. Mean(SE) vitamin B_{12} was higher among women than men: 527.7 (261.6) pg/mL vs 423 (234.6) pg/mL, p<0.0001
			n=1,307 women ≥60y	6.6% women		
Mexico	ENSANUT (38, 51)	Deficiency: <200 pg/mL	N=2, 678 children 1-5 y	1.9%	N/A	Odds of vitamin B_{12} deficiency was lower for children living the south compared to living in the center (OR: 0.22, 95% CI: 0.05-0.95) and for children in the second tertile of socioeconomic status compared to the first tertile (OR: 0.07, 95% CI: 0.01-0.58) Odds for vitamin B_{12} deficiency was higher for indigenous

Appendix table 1 (Continued)

				compared to non-indigenous children 8.7(3.2-23.62)
	N=4,275 children 5-12y	2.6 %		OR for vitamin B12 deficiency was 0.27(0.1-0.72) and 0.21 (0.06-0.69) for in the second and third tertile of socioeconomic status, compared to the first. indigenous children (OR=2.7, 95%CI 1.3-5.6).
	N=4,263 women 20-49y	8.5%		The prevalence of vitamin B_{12} deficiency was significantly higher in rural compared to urban areas (13.2% vs 7.4% and indigenous compared to non-indigenous women (18.6% vs 7.4%). The odds of vitamin B_{12} deficiency were higher for women in Center, Mexico City, and the South compared to the north, socioeconomic quintile 5 compared to 1 (0.15, 95% CI: 0.06-0.40),

ENNyS: National Survey of Health and (Nutrition Encuesta Nacional de Nutrición y Salud, Argentina)
ENS: National Nutrition Survey (Encuesta Nacional de Salud, Chile)
ENSIN: National Survey of Nutrition Situation (Encuesta Nacional de Situación Nutricional, Colombia)
ENN: National Nutrition Survey (Encuestra Nacional de Nutrición, Costa Rica)
ENSANUT: National Health and Nutrition Survey (Enscuesta Nacional de Salud y Nutrición, México)

Appendix Table 2 Adult (>19y) subgroup analysis of correlates of natural logarithmically transformed vitamin B_{12} concentrations

Variables	Beta	SE	P value
Socio-demographic			
Sex			<0.0001
Male	-0.06	0.02	<0.0001
Female	Reference		
Age, years	0.01	0.001	<0.0001
Age Categories			<0.0001
20-29	-0.05	0.02	0.01
30-39	Reference		
40-49	0.05	0.02	0.01
50-59	0.10	0.03	0.001
Life stage			
Pregnant women	-0.32	0.05	<0.0001
WRA (12-49y) vs. Women non WRA	-0.10	0.04	0.01
Area			0.63
Urban	Reference		
Rural	0.01	0.02	0.63
Region			<0.0001
Highlands	Reference		
Coast	0.21	0.02	<0.0001
Amazon	0.07	0.02	0.0003
Galápagos	0.28	0.06	<0.0001
Economic status index			0.05
Q1	Reference		
Q2	0.003	0.03	0.92
Q3	-0.002	0.03	0.96
Q4	-0.034	0.03	0.18

Appendix table 2 (Continued)

Q5	-0.07	0.03	0.01
Ethnicity			<0.0001
Indigenous	-0.04	0.03	0.11
Appendix Table 2. (Continued)			
Afro-Ecuadorian	0.18	0.04	<0.0001
Montubio	0.07	0.04	0.06
Mestizo, white, and others	Reference		
Level of Education			0.0001
None/incomplete primary school	Reference		
Primary school/incomplete secondary	-0.08	0.04	0.03
Secondary school	-0.11	0.04	0.01
Greater than secondary school	-0.16	0.04	0.0001
Anthropometric			
BMI (kg/cm^2)	0.003	0.001	0.01
BMI category			0.0003
Underweight (<18.5 kg/m^2)	0.10	0.06	0.11
Normal (18.5 - <25 kg/m^2)	Reference		
Overweight (25 - <30 kg/m^2)	0.02	0.01	0.29
Obese (≥30kg/m^2)	0.08	0.02	<0.0001
Overweight (≥25 kg/m^2 vs not)	0.03	0.01	0.01

Appendix Table 3 Children (≤19y) Correlates of natural logarithmically transformed vitamin B_{12} concentrations

Variables	Beta	SE	P value
Socio-demographic			
Sex			<0.0001
Male	-0.12	0.02	<0.0001
Female	Reference		
Age, years	-0.02	0.003	<0.0001
Life stage			
Pregnant women	-0.27	0.17	0.03
WRA (12-49y) vs. Women non WRA	-0.10	0.03	0.001
Area			0.12
Urban	Reference		
Rural	-0.04	0.03	0.12
Region			<0.0001
Highlands	Reference		
Coast	0.21	0.03	<0.0001
Amazon	-0.06	0.02	0.01
Galápagos	0.29	0.08	0.001
Economic status index			0.66
Q1	Reference		
Q2	0.00	0.03	0.92
Q3	-0.02	0.03	0.51
Q4	0.02	0.04	0.60
Q5	-0.02	0.03	0.49

Appendix Table 3 (Continued)

Ethnicity			<0.0001
Indigenous	-0.11	0.04	0.003
Afro-Ecuadorian	0.18	0.06	0.003
Montubio	0.14	0.04	0.001
Mestizo, white, and others	Reference		
Level of Education			0.43
None/incomplete primary school	Reference		
Primary school/incomplete secondary	-0.08	0.32	0.81
Secondary school	-0.15	0.32	0.64
Greater than secondary school	-0.11	0.33	0.74
Anthropometric			
BMI z-score Category			0.62
Underweight (z < -2)	-0.06	0.09	0.49
Normal (z ≥ -2, <1)	Reference		
Overweight (z >1, <2	-0.03	0.03	0.27
Obese (z ≥ 2)	0.01	0.05	0.83
Overweight or obese (z ≥ 1) vs not	-0.02	0.03	0.47

Appendix Table 4 Adult subgroup correlates of vitamin B_{12} deficiency and insufficiency

Variable	Vitamin B_{12} deficiency (<148 pmol/L)				Vitamin B_{12} insufficiency (<221 pmol/L)			
	n/N	OR	95% CI	P	n/N	OR	95% CI	P
Socio-demographic								
Sex	663/10947			0.72	3092/10947			<0.0001
Male	239/3366	1.05	(0.81, 1.37)	0.72	1118/3366	1.32	(1.16, 1.51)	<0.0001
Female		Reference			1974/7581			
Age, years		0.99	(0.98, 1)	0.16		0.98	(0.97, 0.99)	<0.0001
Age categories				<0.0001				<0.0001
20-29	263/3762	1.06	(0.8, 1.41)	0.68	1212/3762	1.19	(1.02, 1.39)	0.30
30-39	220/3794	Reference			1078/3794			
40-49	132/2642	0.64	(0.45, 0.91)	0.01	646/2642	0.83	(0.7, 0.99)	0.04
50-59	48/749	1.07	(0.68, 1.69)	0.77	156/749	0.7	(0.54, 0.91)	0.01
Life stage								
Pregnant women	59/236	6.03	(3.63, 10.01)	<0.0001	147/236	3.9	(2.58, 5.9)	<0.0001
WRA (12-49y) not pregnant	331/6906	5.66	(3.42, 9.38)	0.002	1752/6906	1.51	(1.08, 2.12)	0.02
Area[‡]				0.70				0.03
Urban	421/6874	Reference			1990/6874	Reference		
Rural	242/3831	0.97	(0.82, 1.14)	0.70	1102/4073	0.91	(0.84, 0.99)	0.03
Region				<0.0001				<0.0001
Highlands	374/5225	Reference			1676/5225	Reference		
Coast	120/3039	0.53	(0.4, 0.71)	<0.0001	601/3039	0.48	(0.41, 0.57)	<0.0001
Amazon	161/2356	1	(0.77, 1.3)	0.97	764/2356	0.85	(0.72, 1)	0.06
Galápagos	8/327	0.44	(0.2, 0.98)	0.051	51/327	0.42	(0.26, 0.66)	0.0002
Economic status index				0.10				0.01
Q1	147/2567	Reference			682/2567	Reference		
Q2	139/2392	1.3	(0.89, 1.89)	0.18	678/2392	1.2	(0.97, 1.49)	0.09
Q3	132/2166	1.3	(0.89, 1.89)	0.18	631/2166	1.3	(1.04, 1.65)	0.02
Q4	131/2012	1.65	(1.15, 2.36)	0.01	586/2012	1.47	(1.19, 1.82)	0.0003

Appendix Table 4 (Continued)

Q5	114/1804	1.44	(0.99, 2.1)	0.06	515/1804	1.39	(1.1, 1.74)	0.01
Ethnicity				0.21				0.001
Indigenous	88/1138	1.03	(0.7, 1.51)	0.88	350/1138	1.01	(0.78, 1.32)	0.93
Afro-Ecuadorian	17/410	0.6	(0.31, 1.16)	0.13	74/410	0.52	(0.37, 0.74)	0.0003
Montubio	15/379	0.6	(0.31, 1.17)	0.13	91/379	0.72	(0.53, 0.99)	0.04
Mestizo, white, and others	543/9020	Reference			2577/9020	Reference		
Level of Education				0.003				0.01
None/incomplete primary school	11/284	Reference			60/284	Reference		
Primary school/incomplete secondary	263/4527	3.47	(1.65, 7.3)	0.001	1237/4527	1.47	(1, 2.17)	0.05
Secondary school	249/4016	3.73	(1.76, 7.9)	0.0006	1159/4016	1.53	(1.02, 2.29)	0.04
Greater than secondary school	140/2120	4.41	(2.03, 9.56)	0.0002	636/2120	1.86	(1.23, 2.82)	0.003
Anthropometric								
BMI		0.98	(0.96, 1.01)	0.20		0.99	(0.98, 1)	0.06
BMI Category				0.48				0.02
Underweight (<18.5 kg/m^2)		1.28	(0.46, 3.55)	0.64		0.70	(0.37, 1.36)	0.29
Normal (18.5 - <25.0 kg/m^2)		Reference				Reference		
Overweight (25.0 - <30.0 kg/m^2)		1.06	(0.79, 1.44)	0.70		1.07	(0.92, 1.24)	0.41
Obese (≥30.0 kg/m^2)		0.81	(0.55, 1.19)	0.27		0.82	(0.68, 0.97)	0.02
Overweight or obese (≥25.0 vs not)		0.96	(0.73, 1.27)	0.78		0.99	(0.86, 1.13)	0.82

‡Estimates for area are not weighted

Appendix table 5 Child (≤ 19y) of vitamin B_{12} deficiency and insufficiency

Variable	Vitamin B_{12} deficiency (<148 pmol/L)				Vitamin B_{12} insufficiency (<221 pmol/L)			
	n/N	OR	95% CI	P	n/N	OR	95% CI	P
Socio-demographic								
Sex	307/5205			0.002	1789/5205			<0.0001
Male	197/2487	1.88	(1.27, 2.78)	0.002	990/2487	1.71	(1.44, 2.03)	<0.0001
Female		Reference			799/2718			
Age, years		1.07	(1.01, 1.13)	0.02		1.11	(1.08, 1.14)	<0.0001
Life stage								
Pregnant women	05//39	9.16	(1.94, 43.26)	0.01	19/39	4.08	(1.45, 11.51)	0.34
WRA (12-49y) not pregnant	90/2090	1.66	(0.78, 3.54)	0.19	658/2090	1.64	(1.22, 2.21)	0.01
Area‡				0.81				
Urban	180/3085	Reference			1053/3085	Reference		
Rural	127/2120	1.03	(0.81, 1.3)	0.81	736/2120	1.03	(0.91, 1.15)	0.66
Region				0.22				<0.0001
Highlands	156/2283	Reference			852/2283	Reference		
Coast	51/1507	0.65	(0.42, 1.01)	0.05	346/1507	0.52	(0.42, 0.64)	<0.0001
Amazon	90/1232	1.02	(0.71, 1.46)	0.93	547/1232	1.17	(0.94, 1.45)	0.15
Galápagos	10/183	0.77	(0.32, 1.84)	0.56	44/183	0.52	(0.3, 0.89)	0.02
Economic status index				0.66				0.70
Q1	73/1232	Reference			424/1234	Reference		
Q2	72/1201	1.48	(0.89, 2.46)	0.13	410/1201	0.98	(0.72, 1.32)	0.87
Q3	69/1084	1.30	(0.76, 2.23)	0.34	404/1084	1.15	(0.86, 1.55)	0.35
Q4	54/914	1.15	(0.66, 1.99)	0.62	300/914	0.94	(0.69, 1.29)	0.70
Q5	39/771	1.11	(0.63, 1.96)	0.72	251/771	1.05	(0.76, 1.46)	0.76
				0.63				0.00

Appendix table 5 (Continued)

Ethnicity								
Indigenous	42/438	1.27	(0.77, 2.09)	0.35	237/538	1.54	(1.13, 2.1)	0.01
Afro-Ecuadorian	9/167	0.97	(0.44, 2.16)	0.95	41/167	0.65	(0.41, 1.04)	0.07
Montubio	7/182	0.69	(0.29, 1.67)	0.41	39/182	0.53	(0.34, 0.84)	0.01
Mestizo, white, and others	249/4318	Reference			1472/4318	Reference		
Level of Education								0.86
None/incomplete primary school	0/5	Reference			1//5	Reference		
Primary school/incomplete secondary	285/4936	--*	--	--	1678/4936	1.26	(0.13, 12.07)	0.84
Secondary school	16/172	--	--	--	73/172	1.51	(0.15, 15.11)	0.73
Greater than secondary school	6//92	--	--	--	37/92	1.37	(0.13, 14.19)	0.79
Anthropometric								
BMI z-score Category				0.02				0.37
Underweight (z < -2)		1.04	(1.006, 1.08)	0.18	18/60	1.46	(0.67, 3.22)	0.34
Normal (z ≥ -2, <1)		2.6			1124/3437	Reference		
Overweight (z >1, <2		Reference	(0.68, 9.95)	0.16	315/852	1.21	(0.95, 1.55)	0.12
Obese (z ≥ 2)		1.39	(0.85, 2.28)	0.19	113/338	1	(0.67, 1.49)	0.98
Overweight or obese (z ≥ 1) vs not		1.17	(0.75, 1.82)	0.49	428/1190	1.14	(0.92, 1.41)	0.25

‡Area estimates are not weighted

*Unreliable estimates because of small sample size of reference category

Appendix tables 6a-c. Adult (>19y) multivariate models.

Appendix table 6a Adult-only multivariate model for natural logarithmically transformed vitamin B_{12}

Variable	F value	p-value	Beta	SE	p-value
Sex	25.280	<0.0001			
Male			-0.07	0.01	<0.0001
Female			Reference		
Age, years	32.130	<0.0001	0.004	0.001	<0.0001
Ethnicity	5.48	0.001			
Indigenous			0.02	0.03	0.50
Afro-Ecuadorian			0.12	0.04	0.001
Montubio			-0.03	0.04	0.44
Mestizo, white, and others			Reference		
Region	37.27	<0.0001			
Highlands			Reference		
Coast			0.21	0.02	<0.0001
Amazon			0.06	0.02	0.002
Galápagos			0.27	0.06	<0.0001
Level of Education	2.66	0.05			
None/incomplete primary school			Reference		
Primary school/incomplete secondary			-0.04	0.04	0.34
Secondary school			-0.06	0.04	0.16
Greater than secondary school			-0.10	0.04	0.02

Appendix Table 6b Adult-only multivariate model for vitamin B_{12} deficiency

Variable	OR	95% CI		p-value
Age category				<0.0001
20-29	1.02	0.76	1.35	0.9188
30-39	Reference			
40-49	0.65	0.46	0.92	0.0145
50-59	1.16	0.72	1.86	0.542
Region				<0.0001
Coast	0.53	0.40	0.71	<0.0001
Amazon	1.01	0.78	1.32	0.931
Galápagos	0.43	0.19	0.94	0.0352
Highlands	Reference			
Level of Education				0.0033
None/incomplete primary school	Reference			
Primary school/incomplete secondary	3.53	1.65	7.56	0.0012
Secondary school	3.97	1.82	8.65	0.0005
Greater than secondary school	4.54	2.03	10.17	0.0002

Appendix Table 6c Adult-only multivariate model for vitamin B_{12} insufficiency

Variable	OR	95% CI		p-value
Sex				<0.0001
Male	1.39	1.21	1.58	<0.0001
Female	Reference			
Age category				<0.0001
20-29	1.16	1	1.35	0.06
30-39	Reference			
40-49	0.83	0.69	0.99	0.04
50-59	0.70	0.54	0.91	0.01
Area				
Urban	Reference			
Rural	0.74	0.63	0.87	0.0003
Region				<0.0001
Coast	0.45	0.37	0.54	<0.0001
Amazon	0.97	0.82	1.16	0.76
Galápagos	0.45	0.28	0.71	0.001
Highlands	Reference			
Ethnicity				<0.0001
Indigenous	0.86	0.66	1.14	0.29
Afro-Ecuadorian	0.61	0.43	0.87	0.01
Montubio	1.09	0.78	1.51	0.62
Mestizo/white/others	Reference			

Appendix tables 7a-c Children-only (≤19y) multivariate models.

Appendix table 7a Children-only multivariate model for natural logarithmically transformed vitamin B_{12}

Variable	F value	p-value	Beta	SE	p-value
Sex	48.790	<0.0001			
Male			-0.13	0.02	<0.0001
Female			Reference		
Age, years	44.070	<0.0001	-0.02	0.003	<0.0001
Region	39.21	<0.0001			
Highlands			Reference		
Coast			0.21	0.03	<0.0001
Amazon			-0.05	0.02	0.01
Galápagos			0.28	0.08	0.001

Appendix table 7b Children-only multivariate model for vitamin B_{12} deficiency

Variable	OR	95% CI		p-value
Sex				0.001
Male	1.94	1.32	2.86	0.001
Female	Reference			0.01
Age, years	1.08	1.02	1.14	0.01

Appendix table 7c Children-only multivariate model for vitamin B_{12} insufficiency

Variable	OR	95% CI		p-value
Sex				<0.0001
Male	1.86	1.56	2.22	<0.0001
Female	Reference			
Age years	1.13	1.09	1.16	<0.0001
Region				<0.0001
Coast	0.49	0.39	0.61	<0.0001
Amazon	1.16	0.93	1.46	0.19
Galápagos	0.51	0.29	0.89	0.02
Highlands	Reference			